507

Rapid neonatal screening of CFTR mutations by heteroduplex analysis with capillary array electrophoresis (HA-CAE) in the population of Castilla-Leon region (Spain)

M.J. Alonso[1,2], M. Durán[1,2], M. Infante[1,2], E. Esteban[1,2], E. Velasco[1,2], I. Fernández[1,2], J. Tellia[1,2], A. Blanco[1,2]
[1]Laboratorio Pediatria. Univ.Valladolid (Spain), [2]IBGM (Instituto de Biología y Genetica Molecular)

Aims: We present a new method to detect mutations in the CFTR gene based in heteroduplex analysis by capillary array electrophoresis (HA-CAE). The purpose is to optimize the time for analysis sample and the accuracy of mutation detection.
CFTR neonatal screening has demostrated to be very important in order to improve the quality of life of the CF patients. A wide range of techniques can be used to identify mutations and polymorphisms in the CFTR gene. These methods can be divided into two groups: mutation specific methods and generic methods, and some of them are time consuming (sequencing ...) or expensives (OLA, ...). The HA-CAE method have demonstrated to be faster and more effective than other classical methods and with a low cost.
The method was developed in a 3100 Abi Prism automatic sequencer with primers labeled with FAM in the 5´position. We analyse exon 10 (containing F508del mutation: 61%of CFTR mutations in our population) and usind the multiplex PCR method we amplify exons 11, 14b and 17b (14%) and 12 and 7 (7.35%) . With these three PCR reactions we can analyse the 82.35% of our CFTR mutations, overcoming the 80% mutation detection rate reccomended by the European Concerted Action of CF.
Conclusion: this is a suitable method for CF neonatal screening in our population.

508

CAP70 as a possible modifier gene of Cystic Fibrosis phenotype

S. Gambardella[1], M. D'Apice[1], C. Bulli[1], P. Borgiani[1], S. Russo[1], F. Lombardi[1], V. Lucidi[2], G. Castaldo[3], A. Amoroso[4], G. Novelli[1]
[1]Department of Biopathology, Tor Vergata, Rome, Italia; [2]Hospital Bambin Gesù, IRCCS, Rome, Italy; 3Dipartimento di Biochemistry , Federico II, Naples, Italia; [4]Genetic service, IRCCS Burlo-Garofolo, Trieste, Italy

Cystic fibrosis (CF; #219700) is a disorder caused by mutations in the CFTR gene, characterized by a wide variability of clinical expressions modulated by "modifier genes". Recently, biochemical and functional analysis of CFTR, revealed that this protein interacts with protein containing multiple PDZ domain (Cftr Associated Protein 70 kDa, N/H Exchanger Regulatory Factor). We studied a human homolog of the mouse PDZK1, CAP70, as a candidate CF modifier gene. CAP70 maps on 1q21 and is organized in 8 exons which encode a mRNA of 2.2Kb. The gene presents three actively transcribed copies, but one of these is translated in a functional protein product. We genotyped CAP70 in a court of CF patients with pancreatic insufficiency (PI=36) and hepatic involvement and pancreatic insufficiency (EI=31). Results were compared with a matched age normal controls (C=35). Genotypes at SNP IVS4 –92 A/G (rs9726178) upstream of the exon 5 were examined by Pyrosequencing technology to determine the allele copies in a triplicate gene assay. This method is able to reveal seven different allele combinations (6A0G; 5A1G; 4A2G; 3A3G; 2A4G; 1A5G; 0A6G). The allelic distribution was different within CF phenotypic classes compared (Chi square test: p=0.012). In particular, 3A3G were underrepresented within the PI CF patients (p=0.008), while 5A1G, 6A0G, 0A6G, 1A5G were overrepresented in this group of patients (p=0.007).
These data strongly suggest a role for CAP70 gene as modifier of the CF phenotype.